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BIOTECHNOLOGY - ORIGINAL ARTICLE

Assessment of the Biodiesel Production Potential of the Amazonian Microalga *Stigeoclonium* sp. B23 under Different Nutritional Conditions

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ABSTRACT

Despite growing interest in microalgae for biodiesel, the quality parameters of biodiesel from Amazonian species remain largely unexplored. The rich biodiversity of the Amazon and the limited studies of its native microalgae make species such as *Stigeoclonium* sp. B23 promising candidates for sustainable and high-quality biodiesel. This study evaluated the effects of different nutritional conditions on fatty acid production and biodiesel quality in the Amazonian microalga *Stigeoclonium* sp. B23. Eight experimental conditions were established using modified BG-11 medium, combining the presence or absence of sodium nitrate with supplementation of sodium acetate (0.82 g L^{-1}) and/or sodium bicarbonate (0.42 g L^{-1}), to assess the impact of nitrogen limitation and alternative carbon sources. Among the tested experiments, the sodium nitrate depletion combined with supplementation of sodium acetate and sodium bicarbonate (Experiment 8) produced the most favorable fatty acid profile for biodiesel synthesis, with a cetane number of 77.1, oxidative stability of 7.07 h, and a saponification value of 127.3 mg g^{-1}. Experiment 8 also showed a balanced composition of saturated (28.6%) and monounsaturated (6.4%) fatty acids. The biodiesel produced met all evaluated quality standards, including cetane number, iodine value, cold filter plugging point, and oxidative stability. These findings demonstrate the strong biotechnological potential of *Stigeoclonium* sp. B23 as a sustainable source of high-quality biodiesel.

KEYWORDS: lipid profile; environmental biotechnology; sustainable energy; nitrogen depletion

Avaliação do Potencial de Produção de Biodiesel da Microalga Amazônica *Stigeoclonium* sp. B23 sob Diferentes Condições Nutricionais

RESUMO

Apesar do crescente interesse em microalgas para a produção de biodiesel, os parâmetros de qualidade do biodiesel proveniente de espécies amazônicas permanecem em grande parte inexplorados. A rica biodiversidade da Amazônia e os estudos limitados sobre suas microalgas nativas tornam espécies como *Stigeoclonium* sp. B23 candidatas promissoras para a produção de biodiesel sustentável e de alta qualidade. Este estudo avaliou os efeitos de diferentes condições nutricionais na produção de ácidos graxos e na qualidade do biodiesel da microalga amazônica *Stigeoclonium* sp. B23. Oito condições experimentais foram estabelecidas utilizando meio BG-11 modificado, combinando a presença ou ausência de nitrato de sódio com suplementação de acetato de sódio (0,82 g L^{-1}) e/ou bicarbonato de sódio (0,42 g L^{-1}), para avaliar o impacto da limitação de nitrogênio e de fontes alternativas de carbono. Entre os experimentos testados, o tratamento com depleção de nitrato de sódio combinado com a suplementação de acetato de sódio e bicarbonato de sódio (Experimento 8) produziu o perfil de ácidos graxos mais favorável para a síntese de biodiesel, com um número de cetano de 77,1, estabilidade oxidativa de 7,07 h e um índice de saponificação de 127,3 mg g^{-1}. O experimento 8 também apresentou uma composição equilibrada de ácidos graxos saturados (28,6%) e monoinsaturados (6,4%). O biodiesel produzido atendeu a todos os padrões de qualidade avaliados, incluindo número de cetano, índice de iodo, ponto de entupimento do filtro a frio e estabilidade oxidativa. Esses resultados demonstram o grande potencial biotecnológico da *Stigeoclonium* sp. B23 como fonte sustentável de biodiesel de alta qualidade.

PALAVRAS-CHAVE: perfil lipídico; biotecnologia ambiental; energia sustentável; depleção de nitrogênio

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INTRODUCTION

For decades, people around the world have relied on non-renewable sources of energy. In line with rapid development, energy consumption from fossil fuels has also increased proportionally (Megura and Gunderson 2022; Azni et al. 2023). Although new petroleum reserves and advanced extraction technologies continue to emerge, the depletion of fossil fuels is no longer the main global concern. The critical issue now lies in the rising levels of atmospheric carbon and their contribution to climate change. Continued reliance on fossil fuels has intensified greenhouse gas emissions, threatening climate stability, biodiversity, and global economies (Ahmad et al. 2021; Azni et al. 2023). In this scenario, developing renewable and low-carbon energy sources has become imperative to ensure energy security and environmental sustainability (Ahmad et al. 2021; Hasnain et al. 2024). Among these, microalgae-based biofuels stand out for their high lipid productivity, rapid growth, and ability to capture CO_2 efficiently without competing with agricultural land, offering a promising alternative for sustainable biodiesel production (Yadav et al. 2016; Azni et al. 2023; Hasnain et al. 2024).

Many studies reinforce the use of microalgae to produce biofuels such as bioethanol, biodiesel, biomethane, hydrogen, and others (Satheesh et al. 2023; Oliveira et al. 2024; Sharma et al. 2025). Specifically, microalgae represent a viable alternative for biodiesel production, as their fast biomass accumulation and elevated lipid yields make them one of the most efficient renewable sources for replacing fossil fuels (Sharma et al. 2025). Biodiesel is a biofuel produced through transesterification reactions, where the chemical breakdown of triglyceride structures occurs. Since biomass production remains the primary challenge in utilizing algal biomass, supplementation with lignocellulosic hydrolysates has been explored as a strategy to enhance metabolite production during the cultivation of algae and microalgae (Oliveira et al. 2024).

Microalgal fatty acids can be efficiently converted into fatty acid methyl esters (FAME), making them a highly valuable feedstock for biodiesel production. Direct transesterification approaches have attracted considerable attention because they allow lipid extraction and FAME synthesis to occur simultaneously, reducing both energy consumption and processing time. This streamlined process enhances overall efficiency and highlights the practical potential of microalgal biomass in sustainable biofuel production (Loh et al. 2021; Oliveira et al. 2023; Sanjurjo et al. 2024). These organisms can produce up to 30 times more oil per unit area than conventional crops and have significantly faster growth than plants like soy and corn, allowing frequent harvests.

Some microalgal species can achieve high lipid contents under specific stress or optimized cultivation conditions. For example, *Chlorella* sp. reached up to 56% dry weight when subjected to UV mutation and 2 mM H_2O_2 treatment (Sivaramakrishnan and Incharoensakdi 2023), while *Nannochloropsis* sp. CCAP211/78 accumulated up to 59% under nitrate depletion and high light intensity (Abdelkarim et al. 2025). Cultivation of microalgae reduces demand for freshwater and farmland, contributes to CO_2 capture, and avoids competition with food production (Osman et al. 2023). Species of microalgae like *Chlorella* and *Scenedesmus* have been identified as particularly promising for biodiesel production (Hawrot-Paw et al. 2021). For optimal biodiesel production, microalgae should have not only high lipid content but also a well-balanced fatty acid profile, with 40–60% saturated fatty acids (C16:0, C18:0) and 25–35% monounsaturated fatty acids (C18:1), while keeping polyunsaturated fatty acids at moderate levels to ensure oxidative stability. This balance is critical for cetane number, cold flow properties, and overall fuel performance, enabling efficient FAME conversion and high-quality biodiesel (Chen et al. 2018; Hawrot-Paw et al. 2021).

Fatty acids with C16–C18 chains, such as palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) are highly desirable for biodiesel due to their oxidative and thermal stability (Hawrot-Paw et al. 2021). The presence of these key fatty acids was confirmed not only in Amazonian microalgae but also in cyanobacteria, highlighting their potential as biodiesel feedstocks (Aboim et al. 2016). Furthermore, subsequent work has shown that cultivation under high nitrate concentrations and increased light intensity significantly enhances total fatty acid content (Aboim et al. 2019), emphasizing the importance of optimizing growth conditions for biofuel production.

Currently, the Amazon biome represents one of the greatest natural resources in Brazil and worldwide. Therefore, the considerable potential of Amazonian biodiversity for biodiesel productivity and quality using eukaryotic microalgae is undeniable. Some species of the genus *Stigeoclonium* have been reported as potential lipid producers for biofuel applications (Ramasamy et al. 2012), however, information regarding biodiesel parameters and quality remains scarce.

Stigeoclonium sp. B23 is a green filamentous microalgae from the Amazon region with notable biotechnological potential, characterized by branched filaments, elongated cylindrical cells, and the ability to form colonies or grow as loose filaments that adapt well to different growth conditions. Previously, *Stigeoclonium* sp. B23 has been reported to accumulate substantial amounts of lipids under nitrogen-deprived conditions together with the production of polyhydroxybutyrate (PHB), a biopolymer with applications in biomaterials (Mourão et al. 2020). Its biomass also presents a promising lipid composition, making it a valuable candidate for biodiesel production and other renewable bioproducts (Mourão et al. 2020), aligning with the growing demand for sustainable and renewable energy sources.

Therefore, the study aims to carry out a detailed analysis of the fatty acid profile of *Stigeoclonium* sp. B23 under different nutritional conditions, using advanced techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and the BiodieselAnalyzer® program to analyze biodiesel quality parameters. The focus is on evaluating the potential of fatty acids, rather than directly analyzing lipid productivity. We provide new information on the fatty acid composition of this Amazonian microalga, highlighting the microalgal potential for biodiesel production and contributing to sustainable development in the Amazon region.

MATERIAL AND METHODS

Strain and growth optimization conditions

The Amazonian *Stigeoclonium* sp. B23 strain (HAMAB accession number 019132), native of state of Amapá (Brazil), was isolated from a puddle of water and used in the present study. The strain was provided by the *Centro de Genômica e Biologia de Sistemas* (CGBS) of the *Universidade Federal do Pará* (Belém, Brazil). The microalgae were grown in 1 L Erlenmeyer flasks of BG-11 medium, maintaining constant light and temperature conditions for 30 days at a controlled room temperature of 25 °C for a photoperiod of 12h dark/12h light (3,000 lx intensity) with a pH 7, and cultivated under various nutritional conditions.

For the optimization of fatty acid production, eight experimental conditions were established using modified BG-11 medium. The cultures were supplemented with sodium acetate ($C_2H_3NaO_2$, 0.82 g L^{-1}) and sodium bicarbonate ($NaHCO_3$, 0.42 g L^{-1}), either individually or combined, and compared to conditions with or without sodium nitrate ($NaNO_3$) depletion. The experimental design comprised: (1) BG-11 control medium; (2) BG-11 + $C_2H_3NaO_2$; (3) BG-11 + $NaHCO_3$; (4) BG-11 + $C_2H_3NaO_2$ + $NaHCO_3$; (5) BG-11 - $NaNO_3$; (6) BG-11 - $NaNO_3$ + $C_2H_3NaO_2$; (7) BG-11 - $NaNO_3$ + $NaHCO_3$ and (8) BG-11 - $NaNO_3$ + $C_2H_3NaO_2$ + $NaHCO_3$. These experimental conditions are summarized in Table 1. The culture in the eight modified BG-11 media were incubated for 30 days at a controlled temperature of 25 °C for a photoperiod of 12h dark/12h light, with a pH of 7 (Aboim et al. 2019).

Total lipid extraction

For the total lipid extraction, 50 mg of lyophilized biomass of *Stigeoclonium* sp. B23 was transesterified with a solution of 1 mL of 2 M KOH:MeOH, stirring for 30 seconds and placing in an ultrasonic bath for 2 minutes at 50 °C. For the extraction of free lipids, 1 mL of 100% hexane was added to react with the transesterified solution, then stirred again and centrifuged at 11,000 x *g* at room temperature for 1 minute. The supernatant (organic phase) was removed and a solution of 6 mol L^{-1} HCl was added to the pellet, then after agitation and centrifugation, 200 µL of dichloromethane and 200 µL of hexane were added again, ending with the last centrifugation step. The extracted lipids were transferred to vial tubes and *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added, placing them in an ultrasonic bath for 10 minutes as the last step of lipid extraction.

Table 1. Experimental media used to optimize fatty acid production for *Stigeoclonium* sp. B23†.

Experiment	Culture Condition
1	BG-11
2	BG-11 + $C_2H_3NaO_2$
3	BG-11 + $NaHCO_3$
4	BG-11 + $C_2H_3NaO_2$ + $NaHCO_3$
5	BG-11 - $NaNO_3$
6	BG-11 - $NaNO_3$ + $C_2H_3NaO_2$
7	BG-11 - $NaNO_3$ + $NaHCO_3$
8	BG-11 - $NaNO_3$ + $C_2H_3NaO_2$ + $NaHCO_3$

†Note: C_2H3NaO_2: Sodium acetate; $NaHCO_3$: Sodium bicarbonate; $NaNO_3$: Sodium nitrate.

+Supplementation; -Depletion.

Total fatty acid profile by Gas Chromatography-Mass Spectrometry (GC-MS)

A Thermo Scientific Trace 1300 gas chromatograph coupled to a Thermo Scientific MS-ISQ Single Quadrupole mass spectrometer with an AI 1310 autosampler was used, equipped with a ZB-5HT capillary column (30m x 0.25mm x 0.1µm). Helium gas was used as the carrier at a flow rate of 1 mL min^{-1} and 1.0µL of sample was injected in splitless mode. The injector operated at 220 °C and the temperature program of the oven started at 50 °C and increased to 200 °C (8 °C min^{-1}) and was held for 1 minute, then increased to 300 °C (15 °C min^{-1}) for 5 minutes, and finally increased to 350 °C (15 °C min^{-1}) and held for 9 minutes. The MS-ISQ operated with an interface at 280 °C, an ionization source at 280 °C, a mass range between 40-1,000 Da, and electron ionization at 70 eV. The fatty acid identifications were performed by comparing the mass spectra with those from the commercial libraries NIST2011, WILEY2009, and FAMES2011 by retention time. The fatty acid concentration was calculated by normalizing the peak area and determining retention times based on the homology between the substances.

Potential properties for biodiesel synthesis based on fatty acid profile

To analyze the potential properties of the fatty acid profile used in biodiesel synthesis, parameters of the biofuel were evaluated using BiodieselAnalyzer® software, Version 2.2, available at: https://www.researchgate.net/publication/272681288_BiodiselAnalyzer_v11_Setup (Talebi et al. 2013; Talebi et al. 2014). The parameters were predicted using empirical equations based on the fatty acid profile values of the microalgae *Stigeoclonium* sp. B23. The obtained parameters were: degree of unsaturation (DU), saponification value (SV), iodine value (IV), cetane number (CN), cold filter plugging point (CFPP), oxidative stability (OS), kinematic viscosity (υ), and density (ρ). Additionally, the data obtained after the prediction of values were compared with the quality parameter regulation standards for biodiesel. In this sense, the National Petroleum Agency (ANP) regulates biodiesel quality in Brazil, the American Society for Experimenting and Materials (ASTM) is the standard that analyzes parameters in the United States, and the European Committee for Standardization (CEN) is the European body that standardizes parameters through EN 14214, which analyzes biodiesel quality on the continent (Masera et al. 2025).

RESULTS

Fatty acid profile of the microalga *Stigeoclonium* sp. B23

Across the eight experimental conditions of BG-11 (Figure 1 and Figure 2), we found mostly the fatty acids C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (α-linolenic acid), and C20:0 (arachidic acid) (Table 2). The fatty acid found in all cultivations was C16:0, with values ranging from 0.7 to 33.9%, except for the cultivation with BG-11 + $NaHCO_3$ (Table 2). In contrast, nitrate depletion, as shown in Experiments 5-8, led to an overall increase in fatty acid production, particularly in saturated (SFA) and polyunsaturated (PUFA) fatty acids (Figure 2, Table 2). In addition, C18:1, C18:2, and C18:3 fatty acids were predominant in this type of cultivation, except in the control (Experiment 1), where C18 fatty acids accounted for only 2.8% (Table 2), and in the $C_2H_3NaO_2$ + $NaHCO_3$ supplementation (Experiment 4), where they represented 13%. Experiment 4 was also notable for the production of the C20:4 ω-6 fatty acid, which shows strong nutraceutical potential and accounted for approximately 20% of the total fatty acid production (Table 2).

When the *Stigeoclonium* sp. B23 microalgae was cultivated under nitrogen depletion in BG-11 – $NaNO_3$ + $C_2H_3NaO_2$ medium (Experiment 6, Figure 2b), the fatty acids C16:0, C18:2, and C18:3 were present in higher amounts with 33.9% (C16:0) and 34.5% (C18:2), except C18:3, which had a very low value of 1.5% (Table 2). In this study, the amounts of SFA, MUFA, and PUFA in $NaNO_3$-containing media were 25%, 25%, and 50%, respectively (Table 2).

Analyzing the cultures separately and evaluating the supplementation with sodium acetate and sodium bicarbonate, a higher concentration of C16:0 (33.9%) is observed in BG-11 – $NaNO_3$ + $C_2H_3NaO_2$ (Experiment 6) with an increase of approximately 48 times compared to

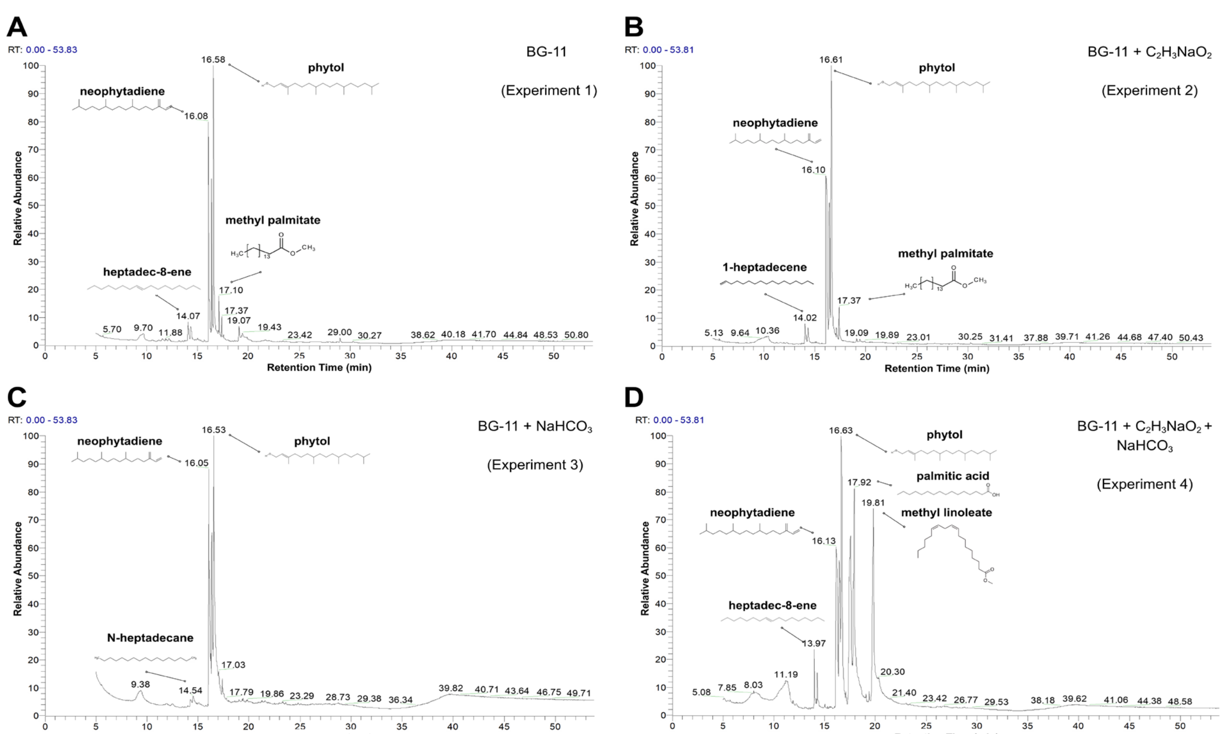

Figure 1. Gas chromatography-mass spectrometry (GC-MS) chromatograms showing the profile of total fatty acids from *Stigeoclonium* sp. B23 cultivated under different nutritional conditions of BG-11 containing $NaNO_3$. A – BG-11 treatment; B – BG-11 supplemented with acetate; C – BG-11 supplemented with bicarbonate; D – BG-11 supplemented with acetate and bicarbonate. Chromatograms display relative abundance versus retention time in minutes. The main identified compounds are indicated in each chromatogram.

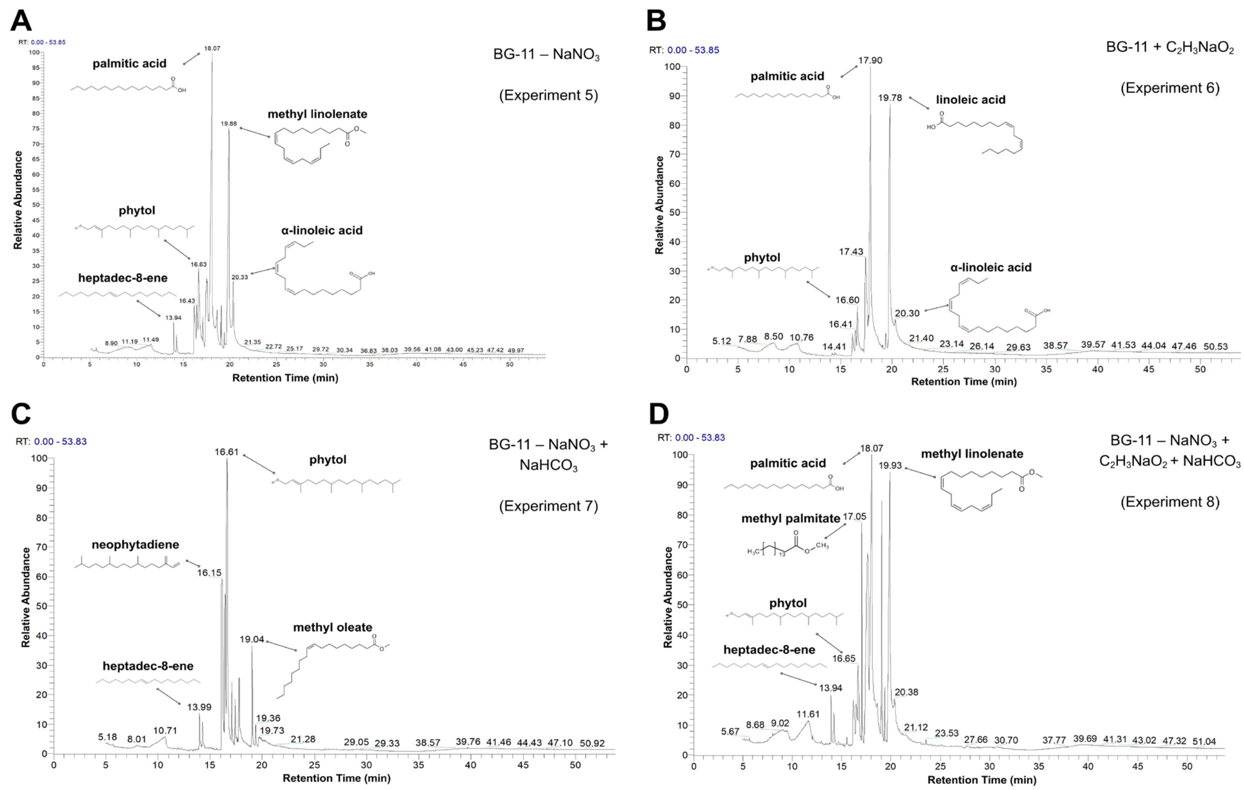

Figure 2. Gas chromatography-mass spectrometry (GC-MS) chromatograms showing the profile of total fatty acids from *Stigeoclonium* sp. B23 cultivated under nitrate-depletion BG-11 conditions. A – BG-11 – Nitrate treatment; B – BG-11 – Nitrate supplemented with acetate; C – BG-11 – Nitrate supplemented with bicarbonate; D – BG-11 – Nitrate supplemented with acetate and bicarbonate. Chromatograms display relative abundance versus retention time in minutes. The main identified compounds are indicated in each chromatogram.

Table 2. Profile of fatty acids (%) determined by lipid extraction in the *Stigeoclonium* sp. B23[†].

Culture *Stigeoclonium* sp. B23	Experiment	Fatty acids								
		SFA	MUFA	PUFA	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4
BG-11	1	4.6	2.8	-	4.6	-	2.8	-	-	-
BG-11 + $C_2H_3NaO_2$	2	0.7	-	-	0.7	-	-	-	-	-
BG-11 + $NaHCO_3$	3	-	-	-	-	-	-	-	-	-
BG-11 + $C_2H_3NaO_2$ + $NaHCO_3$	4	12	-	33.6	12	-	-	13	-	20.6
BG-11 - $NaNO_3$	5	34.2	2.2	32.1	33.5	0.7	2.2	26.9	5.2	-
BG-11 - $NaNO_3$ + $C_2H_3NaO_2$	6	35.2	-	36	33.9	1.2	-	34.5	1.5	-
BG-11 - $NaNO_3$ + $NaHCO_3$	7	10.5	4.6	-	9.3	1.2	4.6	-	-	-
BG-11 - $NaNO_3$ + $C_2H_3NaO_2$ + $NaHCO_3$	8	28.6	6.4	26.3	27.3	1.3	6.4	26.3	-	-

$C_2H_3NaO_2$: Sodium acetate; $NaHCO_3$: Sodium bicarbonate; $NaNO_3$: Sodium nitrate; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

BG-11 + $C_2H_3NaO_2$ (Experiment 2) as shown in Table 2. In the treatment BG-11 – $NaNO_3$ (Experiment 5), there was an increase of 7.4 times (34.2%) compared to the control, indicating that the absence of nitrate in the culture medium leads to a greater accumulation of saturated fatty acids in its composition. In the treatment BG-11 – $NaNO_3$ + $C_2H_3NaO_2$ + $NaHCO_3$ (Experiment 8) more than double the production of C16:0 was obtained (ranging from 12% to 27.3%) when compared to BG-11 + $C_2H_3NaO_2$ + $NaHCO_3$ (Experiment 4). However, no fatty acids were detected in the culture of *Stigeoclonium* sp. B23 in BG-11 + $NaHCO_3$ (Experiment 3) as shown in Figure 1c. The profile of the fatty acids obtained in our experiments is in accordance with the European Standard EN 14214 for biodiesel production, which allows for a maximum amount of C18:3 at ≤ 12%. We found a low quantity of this fatty acid as well as the absence of other polyunsaturated fatty acids (Table 2).

Biodiesel quality parameters calculated from the fatty acid profile

The biodiesel quality parameters varied according to each treatment in modified BG-11 medium is shown in Table 3. All of the cultures with the presence of $NaNO_3$ (Experiments 1, 2, 3, and 4; Figure 1) presented parameters within the evaluated standards for biodiesel quality, such as cetane number (CN) (ranging from 85.9 to 3616.6), iodine value (IV) (ranging from 2.5 to 92.4), cold filter plugging point (CFPP) (ranging from -16.2 °C to -12.6 °C), and oxidative stability (OS).

The iodine values (IV) of all experiments tested were within the EN 14214 limit (≤ 120 g I_2 100 g^{-1}), indicating low unsaturation, except for Experiment 3, which was not detected by the BiodieselAnalyzer® software. The cetane numbers (CN) of all nitrogen-deprived cultures exceeded the minimum requirements of EN 14214 (≥ 47) and ASTM D6751 (≥ 51). Kinematic viscosity and density values also aligned with ABNT and EN specifications, supporting the suitability of *Stigeoclonium* sp. B23 lipids for biodiesel applications.

The CN of all cultures complied with the standards (ranging from 68.0 to 3616.6), the IV also met the standards (ranging from 0g I2.100 g^{-1} to 92.4g I2.100 g^{-1}), as well as the oxidative stability that ranged from 5.8 hours to values considered infinite (‡). Among the $NaNO_3$-depleted cultures (Figure 2), Experiment 5 (– $NaNO_3$) and Experiment 8 (– $NaNO_3$ + $C_2H_3NaO_2$ +$NaHCO_3$) exhibited the most desirable set of quality metrics for high-quality biodiesel. In Experiment 5, *Stigeoclonium* sp. B23 presented a degree of unsaturation (DU) of 66.4, a saponification value (SV) of 143.3 mg g^{-1}, an IV of 65, and a CN of 69.7. The CFPP was -4.7 °C, the OS was 6.2 h, kinematic viscosity (ν) was 2.3 mm^2 s^{-1}, and density (ρ) was 0.6 g cm^{-3}, as shown in Table 3. The parameters indicate good overall fuel characteristics under nitrate depletion.

Experiment 8 (Figure 2d) also performed well, with a DU of 59, SV of 127.3 mg g^{-1}, IV of 53.3, and a notably high CN of 77.1. This treatment achieved the best oxidative stability (OS = 7.07 h) among all conditions in nitrogen depletion and showed kinematic viscosity (ν = 2.2 mm^2 s^{-1}) and density (ρ = 0.5 g cm^{-3}) values consistent with international biodiesel standards. Together, these results highlight the positive effect of nitrate depletion, alone or combined with organic carbon supplementation, on biodiesel-relevant physicochemical properties. The experiments carried out in BG-11 media under $NaNO_3$ depletion (Experiments 5-8) provided the best parameters according to the regulatory standards for biodiesel quality.

DISCUSSION

Cultivation of *Stigeoclonium* sp. B23 under different nutritional conditions significantly influenced its fatty acid profile and biodiesel quality parameters, confirming that nutrient availability is a key factor in lipid metabolism. The most pronounced changes were observed under nitrogen depletion, which promoted the accumulation of storage lipids, while carbon supplementation further modulated the balance between saturated and unsaturated fatty acids. This indicates that both nitrogen limitation and carbon availability act synergistically to enhance lipid biosynthesis and improve biodiesel-related properties.

Nitrogen depletion triggers a marked metabolic reprogramming that redirects carbon flow toward lipid accumulation (Figure 3). As nitrogen availability decreases, the activity of key TCA-cycle enzymes declines, diverting carbon intermediates away from energy production and toward lipid biosynthesis. In this context, acetate enters directly into the acetyl-CoA pathway, enhancing the substrate supply for both saturated and unsaturated fatty acid synthesis, while bicarbonate contributes to carbon fixation and supports the

Table 3. Biodiesel quality parameters based on the fatty acid profile of *Stigeoclonium* sp. B23 in modified BG-11 media.†

Culture *Stigeoclonium* sp. B23	Experiment	DU	SV (mg g^{-1})	IV (g I_2 100 g^{-1})	CN	CFPP (°C)	OS (h)	υ (mm^2 s^{-1})	ρ g cm^{-3}
BG-11	1	2.87	15.8	2.5	389.2	-15	‡	1.1	0
BG-11 + $C_2H_3NaO_2$	2	0	1.5	0	3616.6	-16.2	‡	1	0
BG-11 + $NaHCO_3$	3	ND	ND	ND	ND	ND	ND	ND	ND
BG-11 + $C_2H_3NaO_2$ + $NaHCO_3$	4	67.3	90.3	92.4	85.9	-12.6	11.6	1.7	0.4
BG-11 – $NaNO_3$	5	66.4	143.3	65	69.7	-4.7	6.2	2.3	0.6
BG-11 – $NaNO_3$ + $C_2H_3NaO_2$	6	72	148.6	66.6	68.0	-3.8	5.8	2.4	0.6
BG-11 – $NaNO_3$ + $NaHCO_3$	7	4.6	32	4.2	215.6	-11.6	‡	1.2	0.1
BG-11 – $NaNO_3$ + $C_2H_3NaO_2$ + $NaHCO_3$	8	59	127.3	53.3	77.1	-5.8	7.07	2.2	0.5
ABNT NBR 14747[1]	-	-	-	-	-	≤19	≥6	3-6	0.8-0.9 (20°C)
EN 14214[2]	-	-	-	≤120	≥47	-	≥6	1.9-6	0.8-0.9 (15°C)
ASTM 6751[3]	-	-	-	-	≥51	-	-	3.5-5	-

DU: Degree of Unsaturation; SV: Saponification Value; IV: Iodine value; CN: Cetane Number; CFPP: Cold filter plugging point; OS: Oxidative stability; υ: Kinematic viscosity; ρ: Density; ND: not determined; (‡) Infinite value. 1 - Associação Brasileira de Normas Técnicas - Norma Brasileira; 2 - European Norm; 3 - American Society for Testing and Materials.

replenishment of precursors feeding these biosynthetic routes. This redirection promotes the accumulation of metabolic intermediates, which subsequently impair interconnected pathways, leading to a progressive reduction in ATP synthesis. The decline in ATP availability further weakens cellular energy status, increasing oxidative pressure as redox balance becomes disrupted. Together, these effects intensify reactive species formation and ultimately compromise cellular integrity (Yang et al. 2018; Kupriyanova et al. 2023).

Acetate is a key precursor for fatty acid synthesis in the chloroplast (Figure 3), where it is elongated into the hydrocarbon chains that compose the lipid backbone (Manning 2022). Beyond this role, acetate also contributes to the methylerythritol phosphate pathway, reinforcing its importance in central metabolic routes, as in the Calvin cycle (Figure 3) (Manning 2022). Sodium bicarbonate supplementation also influenced lipid metabolism. Although $NaHCO_3$ can enhance photosynthetic carbon fixation through the carbon-concentrating mechanism (CCM), its isolated addition resulted in only limited lipid accumulation, consistent with reports for *Coelastrella terrestris* (Sangela et al. 2022).

This finding suggests that bicarbonate alone does not substantially promote fatty acid synthesis but may act synergistically with acetate to increase triacylglycerol (TAG) accumulation (Pancha et al. 2015; Yang et al. 2018; Kupriyanova et al. 2023). Under nitrogen stress, microalgae typically redirect carbon flux toward TAG accumulation while downregulating protein and chlorophyll synthesis, a

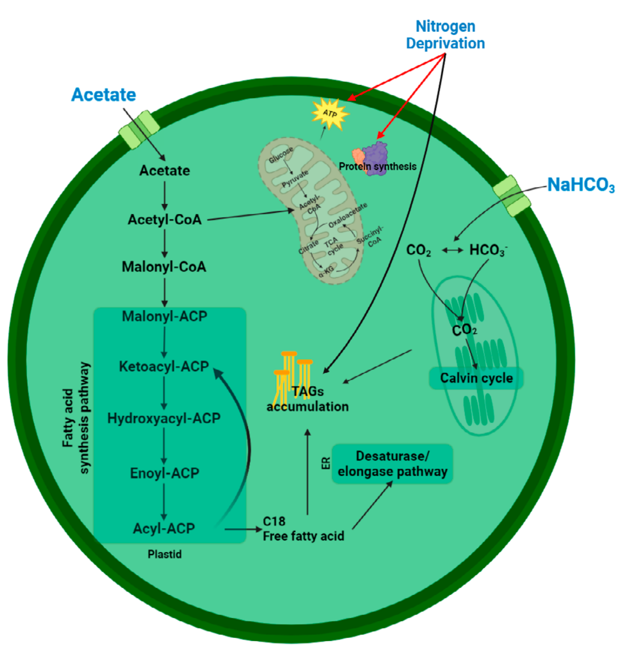

Figure 3. Illustration of lipid biosynthesis pathway during acetate supplementation and nitrogen depletion. Black arrows indicate induction of synthesis, and red arrows indicate negatively affected pathways.

metabolic adjustment widely documented across taxa (Msanne et al. 2012; Morales et al. 2021; Kim et al. 2023). As shown in the flowchart in Figure 3, acetate uptake and bicarbonate assimilation converge to enhance carbon flow toward TAG biosynthesis and accumulation during nitrogen depletion.

As observed in Experiments 5 to 8, *Stigeoclonium* sp. B23 exhibited a substantial increase in saturated fatty acid (SFA) accumulation, reaching 35.2%, which represents a 7.6-fold rise compared to the control condition. This enhancement surpasses that reported for *Chlamydomonas reinhardtii* cultivated under similar conditions, in which supplementation with 1 g L^{-1} and 2 g L^{-1} of sodium acetate led to approximately twofold increases in SFA content (Yang et al., 2018). Similar patterns have been reported in other microalgae such as *Chlorella sorokiniana* FC6 IITG, which cultivated with sodium acetate (15 g L^{-1}) produced 33.6 % of SFA under heterotrophic culture, a value relatively higher than photoautotrophic (28.2%) and mixotrophic (23.6%) cultures (Kumar et al. 2014). In addition, the amount of PUFA also varied in the presence of sodium acetate: heterotrophic culture (19.6%), mixotrophic (39.5%), when compared to photoautotrophic culture (33.7%).

For high-quality biodiesel with commercial value, it is essential to achieve elevated levels of saturated and monounsaturated fatty acids, particularly palmitic acid (C16:0) and oleic acid (C18:1), while minimizing polyunsaturated fatty acids. Recent studies have shown that under dual stress conditions, such as nitrate depletion combined with salinity, *Scenedesmus* sp. SVMIICT1 can accumulate up to 55% oleic acid (w/w) of total fatty acids, significantly enhancing the biodiesel's oxidative stability and cold-flow properties (Kona et al. 2022).

The biodiesel produced in this study met all evaluated quality standards, including cetane number, iodine value, cold filter plugging point, and oxidative stability. A high cetane number (CN) improves diesel performance by ensuring faster ignition, smoother combustion, and lower nitrogen oxide emissions (Saikia et al. 2023). The CN increased with the higher saturated fatty acid (SFA) content observed in both $NaNO_3$-deprived and $NaNO_3$-containing cultures. This relationship reflects the well-established contribution of long-chain SFAs to ignition quality, which strengthens the overall suitability of the resulting biodiesel for applications requiring stable and efficient combustion. In this context, the more favorable biodiesel quality parameters obtained under nitrogen depletion in Experiments 5 and 8 are consistent with the metabolic adjustment illustrated in Figure 3, where reduced nitrogen availability decreases TCA cycle activity and redirects carbon intermediates toward lipid biosynthesis. This metabolic adjustment favors the accumulation of saturated and monounsaturated fatty acids, explaining the lower iodine numbers, higher cetane numbers, and reduced saponification indices observed in these treatments, which displayed the most favorable balance between these fatty acid classes.

The empirical biodiesel quality parameters obtained in this study, including saponification value, iodine value, and cetane number, were superior to those reported by Kumar et al. (2014) for *Chlorella sorokiniana* FC6 IITG, demonstrating the effectiveness of combining nitrogen depletion with targeted carbon supplementation to optimize biodiesel-relevant metabolic pathways.

CONCLUSIONS

This study highlights the remarkable potential of the Amazonian microalgae *Stigeoclonium* sp. B23 as a sustainable source of lipids for biodiesel production, whose capacity to accumulate energy-rich fatty acids under controlled nutritional conditions reinforces the Amazon region as a reservoir of valuable biotechnological resources. Harnessing native microalgae for biofuel generation offers an environmentally responsible alternative for fossil fuels, aligning renewable energy development with the conservation of one planet's most critical ecosystems. Future studies should deepen the understanding of cultivation optimization to enhance lipid yield while preserving the ecological integrity of the Amazon.

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REFERENCES

Abdelkarim, O.H.; Wijffels, R.H.; Barbosa, M.J. 2025. Microalgal lipid production: a comparative analysis of *Nannochloropsis* and *Microchloropsis* strains. *Journal of Applied Phycology* 37: 15–34.

Aboim, J.B.; Oliveira, D.; Ferreira, J.E.; Siqueira, A.S.; Dall'Agnol, L.T.; Rocha Filho, G.N.; et al. 2016. Determination of biodiesel properties based on a fatty acid profile of eight Amazon cyanobacterial strains grown in two different culture media. *RSC Advances* 6: 109751–109758.

Aboim, J.B.; Oliveira, D.T.D.; Mescouto, V.A.D.; Dos Reis, A.S.; Da Rocha Filho, G.N.; Santos, A.V.; et al. 2019. Optimization of light intensity and $NaNO_3$ concentration in Amazon cyanobacteria cultivation to produce biodiesel. *Molecules* 24: 2326.

Ahmad, I.; Yuzir, A.; Mohamad, S.E.; Iwamoto, K.; Abdullah, N. 2021. Role of microalgae in sustainable energy and environment. *IOP Conference Series: Materials Science and Engineering* 1051: 012059.

Azni, M.A.; Md Khalid, R.; Hasran, U.A.; Kamarudin, S.K. 2023. Review of the effects of fossil fuels and the need for a hydrogen fuel cell policy in Malaysia. *Sustainability* 15: 4033.

Chen, Z.; Wang, L.; Qiu, S.; Ge, S. 2018. Determination of microalgal lipid content and fatty acid for biofuel production. *BioMed Research International* 2018: 1–17.

Hasnain, M.; Munir, N.; Sharif, N.; Dias, D.A.; Abideen, Z.; Rizwana, H.; et al. 2024. Potential of algal biomass and their cultivation for biofuels production as plausible bioresource for economic sustainability. *Korean Journal of Chemical Engineering* 41: 2567–2580.

Hawrot-Paw, M.; Ratomski, P.; Koniuszy, A.; Golimowski, W.; Teleszko, M.; Grygier, A. 2021. Fatty acid profile of microalgal oils as a criterion for selection of the best feedstock for biodiesel production. *Energies* 14: 7334.

Kim, S.; Kim, M.; Chang, Y.K.; Kim, D. 2023. Lipid production under a nutrient-sufficient condition outperforms starvation conditions due to a natural polarization of lipid content in algal biofilm. *Fuel* 339: 126902.

Kona, R.; Katakojwala, R.; Pallerla, P.; Sripadi, P.; Mohan, S.V. 2022. High oleic acid biosynthesis and its absolute quantification by GC/MS in oleaginous *Scenedesmus* sp. SVMIICT1 cultivated in dual stress phase. *Algal Research* 67: 102865.

Kumar, K.; Banerjee, D.; Das, D. 2014. Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. *Bioresource Technology* 152: 225–233.

Kupriyanova, E.V.; Pronina, N.A.; Los, D.A. 2023. Adapting from low to high: an update to CO_2-concentrating mechanisms of cyanobacteria and microalgae. *Plants* 12: 1569.

Loh, S.H.; Chen, M.K.; Fauzi, N.S.; Aziz, A.; Cha, T.S. 2021. Enhanced fatty acid methyl esters recovery through a simple and rapid direct transesterification of freshly harvested biomass of *Chlorella vulgaris* and *Messastrum gracile*. *Scientific Reports* 11: 2720.

Manning, S.R. 2022. Microalgal lipids: biochemistry and biotechnology. *Current Opinion in Biotechnology* 74: 1–7.

Masera, K.; Hossain, A.K.; Griffiths, G. 2025. Achieving biodiesel standards through saturation level optimisation. *Frontiers in Fuels* 3: 1528451.

Megura, M.; Gunderson, R. 2022. Better poison is the cure? critically examining fossil fuel companies, climate change framing, and corporate sustainability reports. *Energy Research & Social Science* 85: 102388.

Morales, M.; Aflalo, C.; Bernard, O. 2021. Microalgal lipids: a review of lipids potential and quantification for 95 phytoplankton species. *Biomass and Bioenergy* 150: 106108.

Mourão, M.M.; Gradíssimo, D.G.; Santos, A.V.; Schneider, M.P.C.; Faustino, S.M.M.; Vasconcelos, V.; et al. 2020. Optimization of polyhydroxybutyrate production by Amazonian microalga *Stigeoclonium* sp. B23. *Biomolecules* 10: 1628.

Msanne, J.; Xu, D.; Konda, A.R.; Casas-Mollano, J.A.; Awada, T.; Cahoon, E.B.; et al. 2012. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae *Chlamydomonas reinhardtii* and *Coccomyxa* sp. C-169. *Phytochemistry* 75: 50–59.

Oliveira, D.T.D.; Mescouto, V.A.D.; Paiva, R.D.J.; Silva, S.R.F.D.; Santos, L.A.B.; Serra, G.M.; et al. 2024. Use of residual lignocellulosic biomass and algal biomass to produce biofuels. *International Journal of Molecular Sciences* 25: 8299.

Oliveira, J.; Costa, E.; Maia Dias, J.; Pires, J.C. 2023. Biodiesel production by biocatalysis using lipids extracted from microalgae oil of *Chlorella vulgaris* and *Aurantiochytrium* sp. *BioEnergy Research* 17: 1080–1089.

Osman, M.E.H.; Abo-Shady, A.M.; Gheda, S.F.; Desoki, S.M.; Elshobary, M.E. 2023. Unlocking the potential of microalgae cultivated on wastewater combined with salinity stress to improve biodiesel production. *Environmental Science and Pollution Research* 30: 114610–114624.

Pancha, I.; Chokshi, K.; Ghosh, T.; Paliwal, C.; Maurya, R.; Mishra, S. 2015. Bicarbonate supplementation enhanced biofuel production potential as well as nutritional stress mitigation in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology* 193: 315–323.

Ramasamy, P.K.; Johncy, K.; MubarakAli, D.; Vijayan, D.; Thajuddin, N.; Gunasekaran, M. 2012. Demonstration of increased lipid accumulation potential of *Stigeoclonium* sp. Kütz. BUM11007 under nitrogen-starved regime: a new source of lipids for biodiesel production. *Journal of Biobased Materials and Bioenergy*, 6: 1–5.

Saikia, N.; Sakunthalai, R.A.; Chakradhar, M.; Masilamani, S.; Maheshwari, M.; Saxena, D. 2022. Effects of high cetane diesel on combustion, performance, and emissions of heavy-duty diesel engine. *Environmental Science and Pollution Research* 30: 61246–61256.

Sangela, V.; Kumar, M.; Choudhary, S.; Gour, V.S.; Meena, M.; Vinayak, V.; et al. 2022. Effect of nitrogen, phosphorus and sodium bicarbonate on lipid production and fatty acid profile in *Coelastrella terrestris*. *Biocatalysis and Agricultural Biotechnology* 45: 102518.

Sanjurjo, C.; Rodríguez, E.; Bartolomé, M.; González, R.; Battez, A.H. 2024. Optimizing the conversion of bio-oil from *Haematococcus pluvialis* to fatty acid methyl esters. *BioEnergy Research* 17: 2374–2383.

Satheesh, S.; Pugazhendi, A.; Al-Mur, B.A.; Balasubramani, R. 2023. Biohydrogen production coupled with wastewater treatment using selected microalgae. *Chemosphere* 334: 138932.

Sharma, A.K.; Jaryal, S.; Sharma, S.; Dhyani, A.; Tewari, B.S.; Mahato, N. 2025. Biofuels from microalgae: a review on microalgae cultivation, biodiesel production techniques and storage stability. *Processes* 13: 488.

Sivaramakrishnan, R.; Incharoensakdi, A. 2023. UV mutagenesis followed by hydrogen peroxide treatment ameliorates lipid production and omega-3 fatty acids levels in *Chlorella* sp. *Algal Research* 74: 103195.

Talebi, A.F.; Tabatabaei, M.; Chisti, Y. 2014. BiodieselAnalyzer: a user-friendly software for predicting the properties of prospective biodiesel. *Biofuel Research Journal* 1: 55–57.

Talebi, A.F.; Mohtashami, S.K.; Tabatabaei, M.; Tohidfar, M.; Bagheri, A.; Zeinalabedini, M.; et al. 2013. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. *Algal Research* 2: 258–267.

Yadav, V.; Michalak, A.M.; Ray, J.; Shiga, Y.P. 2016. A statistical approach for isolating fossil fuel emissions in atmospheric inverse problems. *Journal of Geophysical Research: Atmospheres* 121: 12490–12504.

Yang, L.; Chen, J.; Qin, S.; Zeng, M.; Jiang, Y.; Hu, L.; et al. 2018. Growth and lipid accumulation by different nutrients in the microalga *Chlamydomonas reinhardtii*. *Biotechnology for Biofuels* 11: 40.

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